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Case Report

Oral lichen sclerosus expressing extracellular matrix proteins and IgG4-positive plasma cells

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Abstract

Lichen sclerosus (LS) is a mucocutaneous disease with uncommon oral involvement. The etiology is not yet well understood, but LS has been associated with autoimmune, genetic, and immunological factors. We report a 47-year-old man with LS that exhibited an asymptomatic white plaque with red patches on the maxillary alveolar mucosa extending to the labial mucosa. He had no other skin disease. Positive immunostaining for tenascin and scarcity of fibronectin suggested extracellular matrix reorganization. Elastin immunostaining indicated a reduction of elastic fibers. Immunoexpression of collagen IV in blood vessels and its absence in the epithelial basement membrane, together with diffuse MMP-9 immunoexpression, suggested altered proteolytic activity. Mast cell staining bordering areas of sclerosis indicated a possible role in the synthesis of collagen. IgG4 positivity in plasma cells suggested a role in the fibrogenesis. This is an unusual presentation of oral LS and we discuss immunohistochemical findings regarding cellular and extracellular matrix components.

Keywords: lichen sclerosus et atrophicus; oral cavity; immunohistochemistry; metalloprotein; IgG4.

Introduction

Lichen sclerosus is a chronic inflammatory disease of unknown etiology, which affects the skin and mucous membranes. The disease is seen mainly in white postmenopausal women, involving some anogenital sites in 85-98% of cases and extragenital sites in only 15-20% [1,2]. Involvement of the oral mucosa in the absence of simultaneous genital or skin lesions is also uncommon [3,4]. Despite its idiopathic etiology, associations of LS with autoimmune and sex hormone disorders, genetic and immunological factors [5,6], and infection with possible role of *Borrelia burgdorferi* involvement have been proposed [7,8].

The involvement of extracellular matrix (ECM) components in LS has been investigated [9-12]. The hyaline zone seen in LS seems to be related to changes in collagen fiber alignment related to the interposition of glycoproteins [9,10,13,14], glycosaminoglycans, and proteoglycans [11,15]. Furthermore, the association of these alterations with mechanisms of

collagen fiber synthesis and degradation [16] and absence of the elastic fiber system [10] indicates the occurrence of changes in cell-cell and cell-ECM interactions and in the catabolism of ECM components in LS [12].

The head and neck has more commonly been affected by a fibroinflammatory condition named immunoglobulin G_4–related sclerosing disease that shows a male predominance [17]. It involves lymphoplasmacytic infiltrate, elevated serum IgG4, and IgG4-positive plasma cells and was first described in autoimmune pancreatitis [17,18]. Further, other morphological alterations also include obliterative vasculitis and storiform sclerosis [19].

This study reports a case of oral LS in which remodeling of ECM components and IgG4-positive plasma cells were detected by immunohistochemistry. In addition, the clinical and histological features of the case were compared with cases reported in the literature.

Case synopsis

A 47-year-old man, a smoker and recovering alcoholic, was referred for analysis of an asymptomatic white spot that could not be scraped off. The patient reported no history of trauma. Clinically, the lesion appeared as a well-delimited white spot with red patches resembling telangiectasis and was located in the maxillary alveolar mucosa, extending to the labial frenum (Figure 1A).

The lesion was discovered accidentally and measured approximately 2 x 1.5 cm. Alveolar bone loss was detected radiographically (Figure 1B) and the patient had a recent history of tooth extraction in the region. He was in good health and did not use any medication. The clinical diagnosis was erythroleukoplakia. An incisional biopsy was obtained and microscopic analysis revealed a stratified parakeratinized squamous epithelium of variable thickness exhibiting hydropic degeneration of basal cells and cleft-like subepithelial spaces (Figure 2A). The connective tissue of the lamina propria was acellular and hypocellular and showed either areas of lymphedema (Figure 2A) or collagen homogenization (Figure 2B) that in areas displaced the band-like inflammation (Figure 2C). This inflammation consisted predominantly of lymphocytes although plasma cell plaques were also seen. Perivascular inflammation, obliterative vasculitis (Figure 2D), and basement membrane hyalinization (Figure 2E) were sometimes observed. On the basis of the clinical and histopathological features, the diagnosis of LS was established.

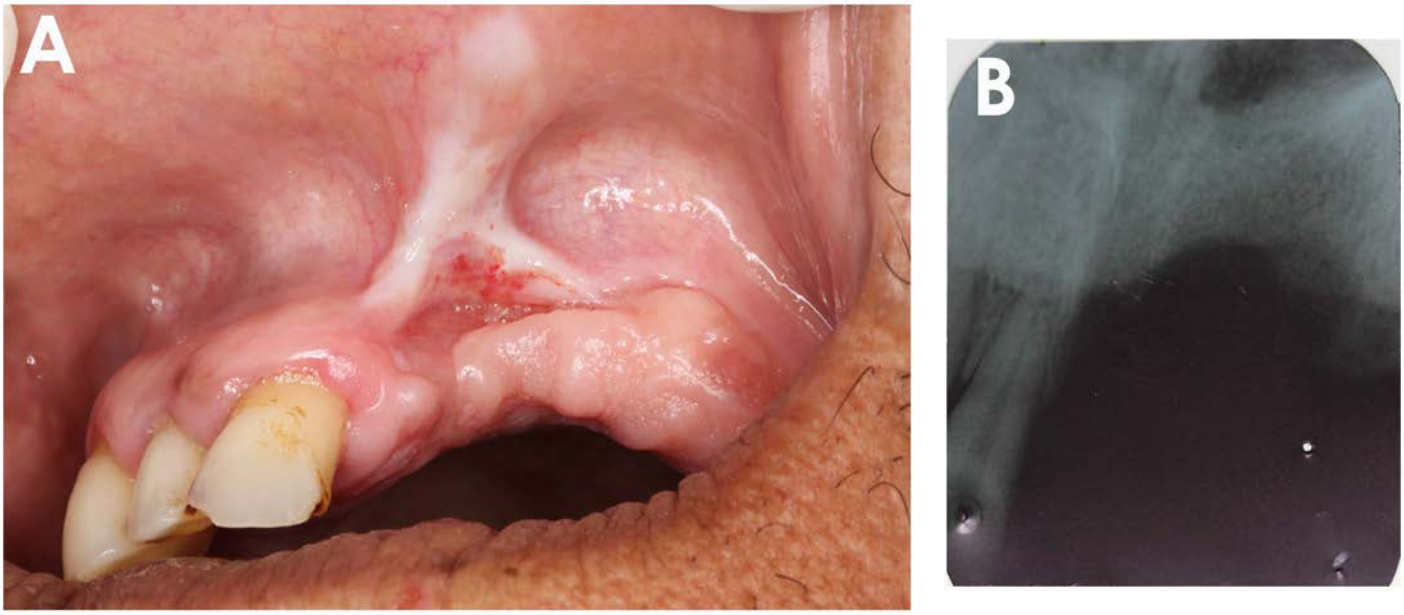

Figure 1. Oral LS characterized by a white patches and red spot resembling telangiectasis

Weigert orcein stain and immunohistochemistry by the immunoperoxidase method for the detection of tenascin, fibronectin, collagen IV, matrix metalloproteinase 9 (MMP-9), mast cells, CD68, CD3, CD45RO, IgG4, p53 and Ki-67 were used to aid understanding of the disease. The results are summarized in Table 1.

Briefly, for immunohistochemistry, the fixed tissue was cut into 4µm sections and the specimens were mounted on silanized glass slides. After deparaffinization, the slides were transferred to 10 mmol/L sodium citrate buffer and heated for 20 min at 95°C for antigen retrieval. Next, the slides were washed in phosphate-buffered saline (PBS) and incubated for 30 min in peroxidase block to quench any endogenous peroxidase activity. After washing in PBS, the slides were incubated for 10 min in serum block and were then incubated with the prediluted primary antibody overnight in a moist chamber. Primary antibody specifications and reaction conditions are listed in Table 1. The slides were rinsed in PBS and incubated for 30 min with the biotinylated secondary antibody. After rinsing in PBS, the slides were incubated with the horseradish peroxidase-streptavidin

complex (DAKO Corporation, Carpinteria, CA, USA) for 30 min. Finally, the reaction was developed with 3,3-diaminobenzidine (DAKO Corporation, Carpinteria, CA, USA) as chromogen and the sections were washed in distilled water and counterstained with hematoxylin.

Histochemical and immunohistochemical analysis revealed an altered distribution of ECM components (Table 2). These changes included a consistent decrease of elastic fibers in areas of sclerosis (Figure 2F), indicating the loss of elastic fibers, and an occasional increase adjacent to the epithelium and site of inflammation. The increased immunostaining for tenascin in the lamina propria, particularly in the area of sclerosis (Figure 3A), corroborates the reorganization of ECM in this lesion. In addition, fibronectin immunoexpression was decreased particularly in lymphedema areas, with the observation of a small increase of fibronectin in the intermediate and deep layers of the lamina propria, corresponding to the band of inflammatory cells (Figure 3B), as well as in focal areas of the lamina propria. Immunostaining for collagen IV was commonly seen in blood vessels, but was not detected in the cleft-like spaces of the epithelial basement membrane. MMP-9 was expressed in epithelial cells, fibroblasts, inflammatory cells, ECM adjacent to the epithelium, and in its deeper region (Figure 3C).

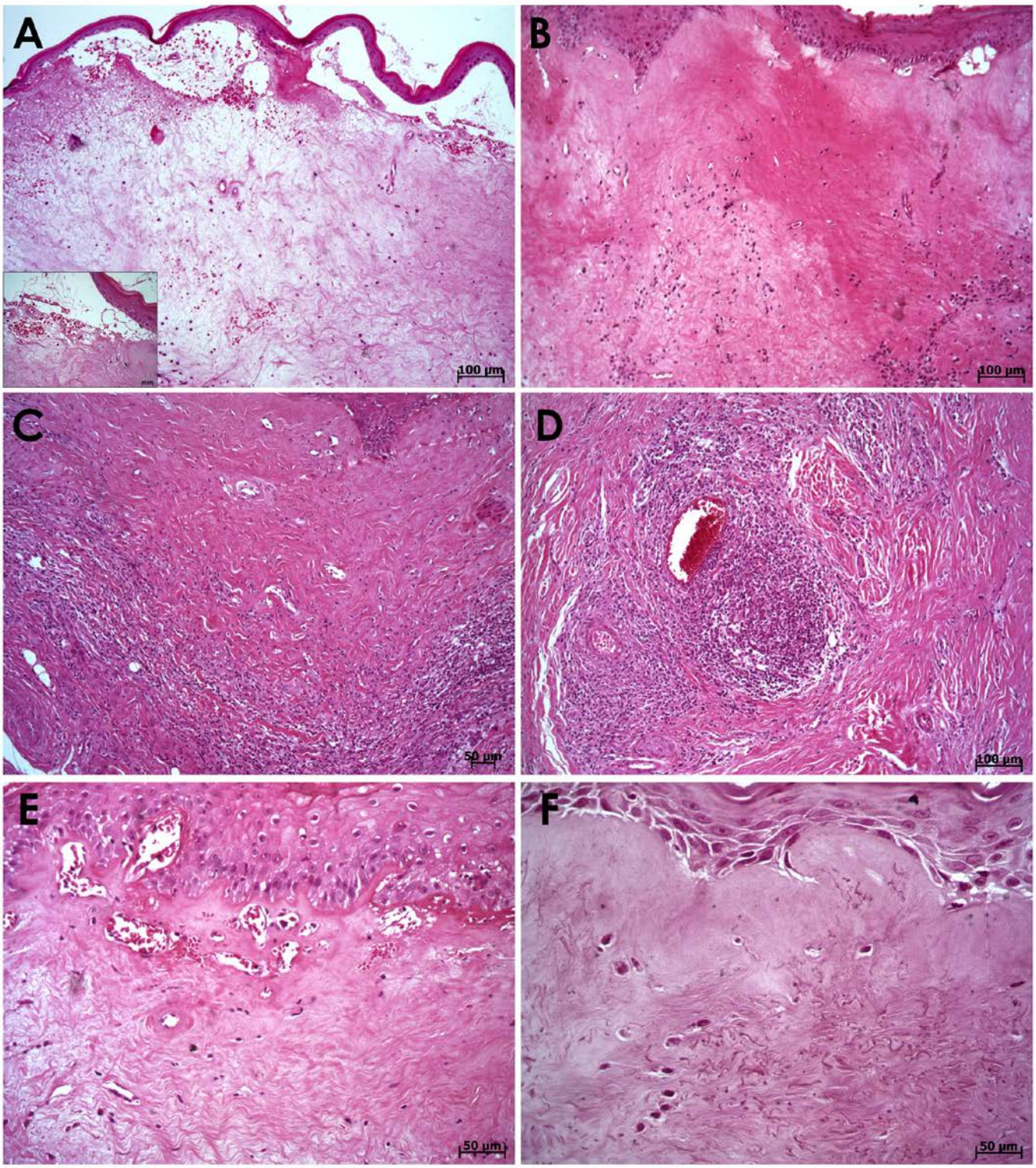

Figure 2. (A) Oral mucosa covered by atrophic epithelium exhibits cleft-like spaces and marked lymphedema in the superficial and deep lamina proprias. (B) Lamina propria shows areas with marked collagen homogenization and other hypocellularity. (C) Deep lamina propria shows collagen displacing the band-like inflammatory infiltrate. (D) Obliterative vasculitis is exhibited. (E) The epithelium displays thick and hyaline basal membrane and collagen homogenization. (F) Marked reduction of elastic fibers is shown in areas of sclerosis.

Intense and diffuse mast cell immunostaining was observed (Figure 3D). Mast cells were irregularly dispersed in the lamina propria, bordering the area of sclerosis. Scarce immunoexpression of CD68 was randomly seen in the mucosa. Focal immunoexpression of CD3 and intense and diffuse immunoexpression of CD45RO were detected in the inflammatory infiltrate. In contrast, positive IgG4 immunostaining was observed in plasma cells and lymphocytes, mainly in areas of vasculitis and, less frequently, in areas of sclerosis (Figure 3E). A low Ki-67 labeling index (Figure 3F) and weak expression of p53 in basal and parabasal cells were also observed in the present case.

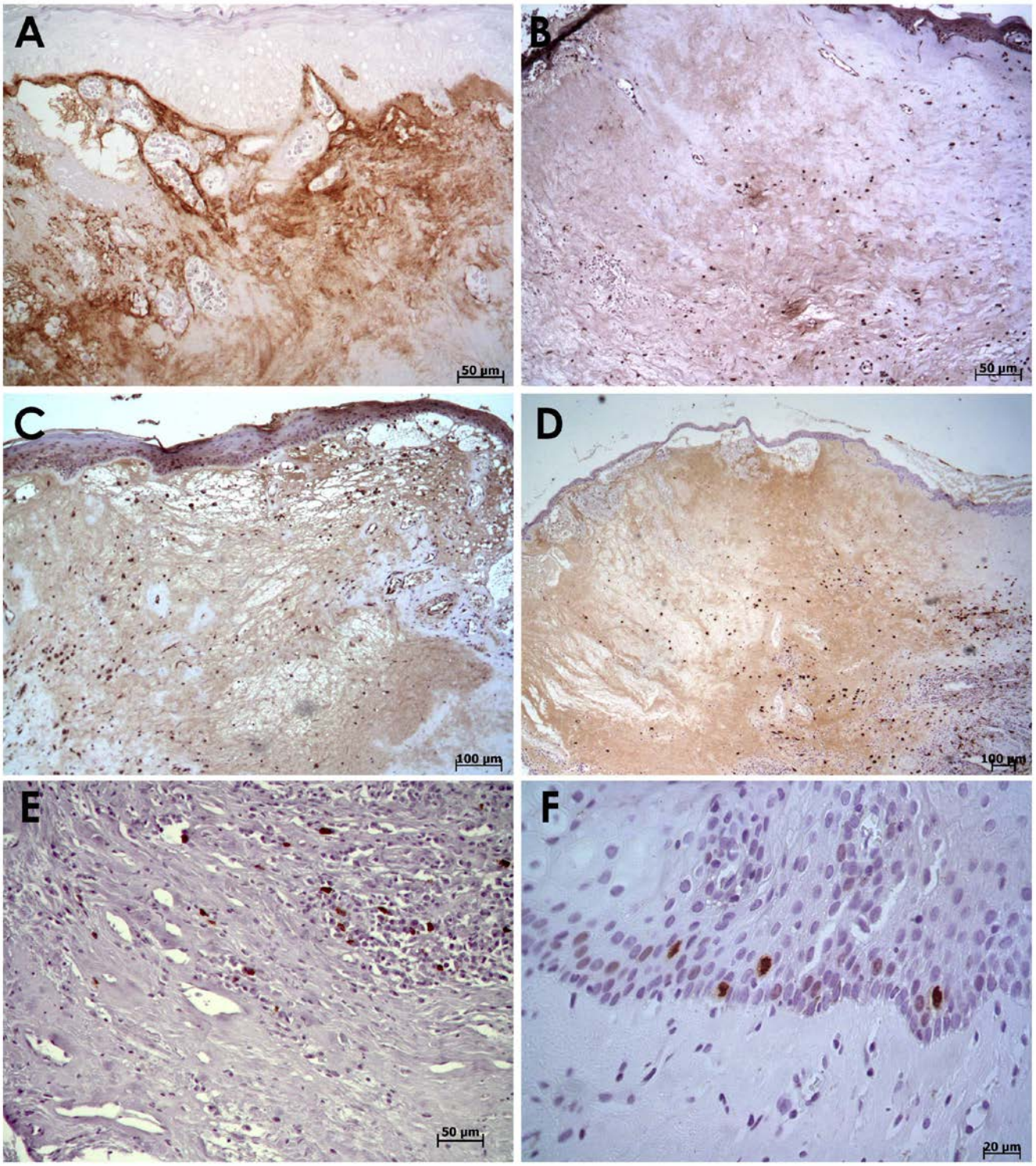

Figure 3. (A) Strong immunostaining of tenascin is shown throughout lamina propria. Note thickness of basement membrane highlighted by the tenascin. (B) Weak immunostaining is present especially in lymphedema areas. (C) Epithelial and stromal cells and collagen fibers weakly immunostained for MMP-9. (D) Diffuse population of mast cells throughout lamina propria (E) Plasma cells and lymphocytes immunostained for IgG4. (F) Rare epithelial cells immunopositive for Ki-67

The oral lesion was excised surgically. After one year of follow-up, the patient remains asymptomatic, without functional or cosmetic concerns, and did not develop any new oral lesions. The patient was also referred to a dermatologist for detailed investigation of skin lesions and did not show any skin or anogenital lesions.

Discussion

Oral LS is uncommon and, to the best our knowledge, nearly 87 cases have been reported in the international literature (PubMed 1957-2014). In addition to the present case, the oral mucosa, with extension to the labial mucosa, was a common oral site [20-22], as well as the vermilion of the lip [22, 23]. Oral LS can also affect the palate [21], gingiva [8,22], and tongue [22,23,25]. It may frequently occur in the absence of skin or genital lesions [22, 25-28]. The present case was an almost 50-year-old man who presented with a white and telangiectatic lesion without any skin or genital lesions. Other clinical features including white flat spots or plaques of variable size have been also reported [1,21,23].

Clinically, the differential diagnosis of oral LS includes lichen planus, leukoplakia, oral submucosal fibrosis, chronic hyperplastic candidiasis, and other white flat mucosal lesions [20]. However, the present case fulfills the morphological criteria of LS reported in previous studies [4,21,22]. The lesion consisted of predominantly reduced squamous epithelium exhibiting hydropic degeneration of basal cells and detachment of the epithelial-connective tissue interface, accompanied by a homogenous acellular zone, underlying chronic inflammation, and areas of lymphedema. Other findings included edema and hyalinization, which are expected to occur after the edematous phase in LS [1,29,30]. We also found vasculitis, which has not yet been noticed by others [4,19,31].

This study showed that tenascin and fibronectin played a significant role in the present case as their expression was altered. This finding is in accordance with those found by Farrel et al [10] in vulvar LS. These authors detected increased immunoexpression of tenascin in the upper dermis corresponding to the area of sclerosis and relatively low expression in the band-like inflammatory infiltrate. Reduced expression of fibronectin was noted in the upper dermis and a slight increase in the intermediate and deep layers of the dermis corresponding to the inflammation area [10]. In the present case, elastic fibers were scarce in the lamina propria, in agreement with previous reports on oral LS [22].

The present results also revealed a significant increase in the immunoexpression of MMP-9, with the observation of a larger number of positive cells in the lamina propria adjacent to the epithelium, suggesting the participation of proteases in the pathogenic process. It is known that most of the proteolytic activity of tumor cells is derived from the adjacent stroma and from immune system cells, as demonstrated for vulvar carcinoma, vulvar intraepithelial neoplasia, and LS [32]. Oliveira et al [12] also observed a larger number of keratinocytes and inflammatory and stromal cells immunopositive for MMP-2, MMP-9, TIMP-1, and TIMP-2 in LS samples when compared to normal skin samples. These authors highlighted that molecular factors involved in the degradation of ECM in LS, such as the expression and secretion of MMPs and their tissue inhibitors, are hallmarks in the progression of this intriguing disease of unknown etiology.

Mast cells trigger the early events of neoplastic progression by the activation of fibroblasts or by ECM remodeling and activation of angiogenesis [33,34]. The intense mast cell immunostaining observed in the present case agrees with these functions, particularly ECM remodeling, suggesting a crucial role of these cells in the synthesis of homogeneous collagen found in this disease. In addition, mast cell degranulation may stimulate cell proliferation, a fact suggesting tryptase-induced proliferation. However, MIB-1+ keratinocytes have also been detected in the absence of mast cells, a finding indicating that the intraepithelial localization of mast cells is not a prerequisite for the proliferation of keratinocytes [35]. A previous study demonstrated a role of these cells in antigen presentation [36].

IgG4-positive plasma cells and lymphocytes were also detected in the present case, particularly in areas of vasculitis and, less frequently, in areas of sclerosis and collagen homogenization. The same changes are observed in IgG4-related sclerosing disease [37]. It is postulated that the infiltrate of IgG4-positive plasma cells coexists with fibrosis. Although this is not well established [38], one may speculate that IgG4-positive cells play a role in fibrogenesis and tissue remodeling in oral LS [38,39]. Further studies including larger series are needed to confirm this hypothesis.

Markers of cell proliferation, such as Ki-67 and tumor suppressor proteins (e.g., p53), are significantly overexpressed in genital LS. Extragenital LS differs significantly from genital LS in terms of cell cycle regulation and proliferation rates. Although cases of malignant transformation of LS, particularly genital LS, have been reported [40], the low Ki-67 labeling index and weak expression of p53 in basal and parabasal cells observed in the present case do not support this hypothesis, in agreement with previous findings [41].

Treatment of oral LS is generally may not be necessary because the disease is usually asymptomatic. However, some patients feel a slight discomfort and pain which can be explained by sclerosis of the lesion [3,24,28]. So far, no effective curative therapies are available for oral LS. Topical application or intralesional injection of corticosteroids has been used successfully in some cases of oral LS [24,29]. The present patient was submitted to surgical excision of the lesion, which was successful after one year of follow-up. Schulten et al [28] also adopted this approach and obtained success after 7.5 years of follow-up.

Finally, this study reported a case of oral LS presenting as a single manifestation of the disease showing that cellular and matrix extracellular components such as mast cells, fibronectin, and tenascin participate on the tissue remodeling of this lesion. However, other studies highlighting the relationship between these components and IgG4 are encouraged. Furthermore, our findings do not support the hypothesis of malignant alteration of oral LS.

Table 1. Primary antibodies (source and clone specification), dilution, antigen retrieval and brand used in immunohistochemistry.

Antibody	Clone	Dilution	Antigen Retrieval	Brand
CD3	F7.2.38	1:50	Citrate buffer pH 6.0	DAKO
CD45RO	UCHL 1	1:50	Citrate buffer pH 6.0	DAKO
CD68	PG-M1	1:100	Citrate buffer pH 6.0	DAKO
Collagen IV	CIV 22	1:50	Citrate buffer pH 6.0	DAKO
Elastin	BA-4	1:100	Trypsin 1%	NovoCastra
Fibronectin	FBN11	1:50	Trypsin 1%	DBS
IgG4	MRQ-44	1:200	Citrate buffer pH 6.0	Monosan
Ki-67	MIB-1	1:100	Citrate buffer pH 6.0	Dako
Mast Cell	AA1	1:50	Trypsin 1%	Dako
MMP-9	Poli	1:50	No recovery	DBS
p53	318-6-11	1:50	Citrate buffer pH 6.0	Dako
Tenascin C	49	1:100	Citrate buffer pH 6.0	Leica

Table 2. Immunostaining profile of the present case of oral lichen sclerosus

Antibody	*Epithelium*	*Upper lamina propria/ area of sclerosis*	*Intermediate lamina propria/ inflammatory infiltrate*	*Deep lamina propria*	*Cell type and/or structure involved*
Elastin	-	-	+a	-	ECM and blood vessels
Tenascin	-	+++b	++b	-	ECM, epithelial basement membrane and blood vessels
Fibronectin	-	+b	++b	-	ECM, lymphocytes and plasma cells/vasculitis
Collagen IV	-	-	-	-	Blood vessels
MMP-9	+b	+b	++b	++b	ECM, fibroblasts, lymphocytes and plasma cells/vasculitis
Mast cell	-	+b	+++b	++b	
CD68	-	+a	+a	+a	
IgG4	-	-	+a	-	Lymphocytes and plasma cells/vasculitis
Ki-67	+a	-	-	-	Basal and suprabasal cells
p53	+a	-	-	-	Basal and suprabasal cells

-, no staining; +, weak staining; ++, moderate staining; +++, strong staining; [a]focal; [b]diffuse. ECM: extracellular matrix.

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